Microautoradiographic Study of Technetium-99m Colloid Uptake by the Rat Liver

Elif Hindie, Nicole Colas-Linhart, Anne Petiet, and Bernard Bok

Department of Biophysics, Faculty of Medecine X. Bichat, Paris, France

A new microautoradiographic technique was developed to study the distribution of ^{99m}Tc-labeled radiopharmaceuticals. Using a thick emulsion, it is possible to get microscopically visible tracks of internal conversion and Auger electrons. The liver uptake of microscopic particles has been thought to occur in Kuppfer cells but no direct evidence has been provided for technetium colloids. Using this method, ^{99m}Tc-labeled colloids were clearly identified in Kupffer cells in the sinusoidal areas of liver. "Track" microautoradiography using a thick emulsion layer may be used on any frozen tissue sections and may provide an important tool to assess the biodistribution of ^{99m}Tc radiopharmaceuticals.

J Nucl Med 29:1118–1121, 1988

Technetium-99m (^{99m}Tc) has been the most widely used radioisotope in human scintigraphic investigation. Therefore, the biodistribution of ^{99m}Tc-labeled molecules or drugs is often relevant and should be studied by microautoradiography. Several drawbacks, however, have prevented the use of this technique. The very short half-life (6 hr) of this radionuclide makes it difficult to obtain good tissue preparations in time for microtome slicing before photographic exposure. Moreover, ^{99m}Tc is often considered as a "pure" gamma emitter. For these reasons, only macroautoradiography has been described. Internal conversion and Auger electrons are emitted in ~10% of all disintegration and can be used to obtain "track" microautoradiographies (*1*). This method is able to detect the whole path of ^{99m}Tc secondary electrons within nuclear emulsions, and has been used on cell smears (*2*) to study the migration of labeled leukocytes (*3*). In the present study, we applied this microautoradiographic track method to liver sections to measure the ^{99m}Tc sulfur colloid (SC) uptake by this organ. The [^{99m}Tc]SC used for hepatic scintigraphy is rapidly cleared from the circulation (10 min) following i.v. injection. The liver uptake is ~80% of the injected activity. Kupffer cells, one type of cell which lines hepatic sinusoids, can fix and phagocyte circulating foreign material (*4*), and are thought to be the main site of ^{99m}Tc uptake (*5*). Direct visualization of ^{99m}Tc colloid inside the Kupffer macrophages, however, has not been performed yet.

Received July 7, 1987; revision accepted Dec. 11, 1987.

For reprints contact: Nicole Colas-Linhart, MD, Dept. of Biophysics, Fac. X. Bichat, 16 rue Huchard 75018 Paris, France.

MATERIAL AND METHODS

In and Ex Vivo Experiments

Three colloidal solutions were prepared. The first one was a [^{99m}Tc]SC solution.

The activity was 500 μCi (18.5 MBq) in 1.4 ml (TCK1 KIT CIS, Gif sur Yvette, France). The average diameter of the colloidal particle was ~3,000 Å (*5*). The second was a colloidal pink dye solution (Le Franc-Bourgeois, "Rose Tyrien", le Mans, France). The third one was simply a mixture of the same ^{99m}Tc colloidal solution and one drop of the pink dye.

A carotid catheter was placed in 12 male Wistar rats (weighing between 400 and 500 g) under urethan anesthesia. Twenty minutes after the colloidal injection, the animals were exsanguinated and the liver was immediately fixed in situ by direct injection into the carotid artery of 15 ml formol 10% in NaCl 0.9%.

The animals were divided into three groups. In the first group, each animal received 1.4 ml of [^{99m}Tc]SC solution. In the second group, each animal was injected with the radioactive solution containing one drop of pink dye. In the third group, only pink dye was injected. This group was not subjected to autoradiography.

Microautoradiography

After 30 min, the liver was excised and one piece was fixed in saline formol for another 30 min. Frozen sections 10 μm thick were put on gelatin coated slides (gelatin coat 1-μm thickness) which were then dipped in 95% ethanol for 5 min, and then washed in double distilled water and allowed to dry at room temperature.

The track autoradiographic technique applied to white blood cells has been previously described (*2*), but was now applied to frozen tissue sections. Briefly, the nuclear emulsion (K5 Ilford) was melted and diluted with an equal volume of

double distilled water, to which a drop of glycerol had been added. Good mixing occured in 10 min at 45°C. Five hundred microliters of the emulsion was poured onto each slide and homogenously smeared to obtain a coating 25-μm thick. The slides were allowed to dry by a temperature gradient of 0.32°C/cm, and placed in individual boxes for the time of exposure which varied between 14 and 21 hr.

The preparations have been submitted to an activated procedure (*6*). The slides were immersed for 20 min at 15°C ± 1°C in a gold solution containing 0.004% KAuCl4, 0.05% KSCN, 0.06% KBr. After rapid rinsing in a 5% NA_2SO_4 solution, the slides were developed for 20 min in a bath of amidol developer (pH = 6.4) (*7*) at 15°C ± 1°C and fixed in 33% sodium hyposulfite until the emulsion coat became transparent (~30 min). The entire technique was performed in complete darkness.

After washing in water, tissue sections obtained from the first series of rats (not injected with dye), were stained with 10% Giemsa and then dried. Tissue sections from the second and third group were not stained. For autoradiographic controls and background, a section from a rat not injected with radioactivity and gelatin coated slides were submitted to autoradiography.

RESULTS

In group one, microscopic examination of the sections showed the ^{99m}Tc-colloidal uptake areas (Fig. 1). Approximately four uptake areas were recovered by each hepatic lobule section. Radioactive areas were preferentially localized near sinusoids between hepatic parenchymal cords to one side of portal tributaries. Using high magnification (×500) tracks were clearly visible. The apparent lengths of visualized tracks, however, varied considerably. In addition to the actual variations in track lengths, the obliqueness may differ considerably between tracks. Optical planes of hepatic architecture and materialized tracks were different: their simultaneous visualization in the same image plane was often difficult (Fig. 1C). Outside these ^{99m}Tc-colloid uptake areas only a few "grains" were recovered in hepatic lobule central regions. These grains were nonspecific of track localizations (Fig. 1D).

In Figure 2, the results of the second group are presented. There was good evidence by simple microscopic examination that phagocytosis is located in Kupffer cells, since pink areas were related to these cells (×500). About three phagocytosed dye particles were observed by hepatic lobule section (Fig. 2A). After MAR, pink dye particle uptake was usually associated with "tracks" (Fig. 2B, C) (×500). In spite of overall good agreement, some dissociated track areas and pink spots could also be observed separately. In Fig. 2D the same uptake area on two different optical planes is shown. Tracks appeared gradually when modifying the focal plane of the microscope. Background activity assessed on control slices with and without tissue section was quite low.

DISCUSSION

In spite of the increasing number of molecules and living cells labeled with ^{99m}Tc, very few authors have employed autoradiography to study their biodistribution. Some examples have been given using ^{99m}Tc-labeled phosphate derivatives, directly showing uptake of this radiotracer in skeletal and cardiac tissues (*8,9*). Two techniques may be utilized.

The first one is a macro-autoradiographic technique using x-ray film which shows local accumulation of the radiopharmaceutical (*10*). The second one makes use of a monolayer nuclear emulsion to detect low-energy electrons. However, the "grains" obtained are not easily differentiated from background noise and the exact site of emission (*11*) cannot always be derived accurately from the image obtained. Some artifacts can be seen on "grains" micro-autoradiograms, due to dust, thermal or mechanical shock, chemical agents, or light spots. In addition, exposure to radiation may come from background radioactivity such as ^{40}K in glass material, ^{14}C in gelatin or cosmic rays (*12*).

The technique described here makes use of a thicker nuclear emulsion which results in a noticeable absorption of high-energy (120–142 keV) conversion electrons as well as lower energy (15–21 keV) Auger electrons emitted during transition of ^{99m}Tc (*13*). Gold activation of the sublatent image obtained better visualization of the whole path of electrons and identification of emission points possible. Most artifacts and "noise" radiation exposure are avoided using the "track" method. Indeed, background activity assessed on control slides devoid of tissue sections was quite low. Thus the "track/background" or signal/noise ratio in the uptake areas is much higher using the "track" method.

Besides the basic principle of using a thick emulsion layer to get "tracks" of the secondary electrons, some modifications of usual autoradiographic techniques were required due to the fast decay ($T_{1/2}$ = 6.0 hr) of ^{99m}Tc. Frozen sections were preferred, although it is often more difficult to observe minute architectural details. The exposure time selected (18 hr) provided the best signal/noise ratio.

The spatial resolution of this technique is obviously limited by the lengths of the tracks, which may vary considerably, according to the energy of the electrons and also to the obliqueness of the path followed within the emulsion. This is clearly seen in high magnification (×500) pictures (Fig. 1C). Maximal lengths observed were ~12 μm.

To validate this method in living tissues, we chose an apparently simple model. The phagocytizing properties of Kupffer macrophage has been well established in a wide variety of particles (*14*). It is generally (*5*) believed that phagocytosis by Kupffer cells is the basis of liver uptake of [^{99m}Tc]SC. Yet, there is no direct evidence of this mechanism.

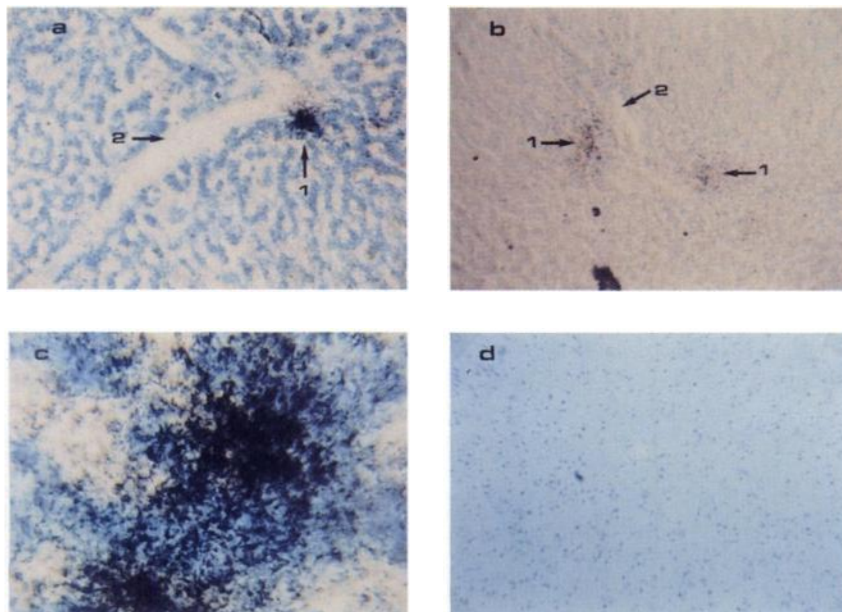

FIGURE 1
Results of ^{99m}Tc colloids MAR in rat liver. The four photographs (a,b,c,d) were obtained after MAR on frozen liver sections following ^{99m}Tc colloidal injection. a and b: Radioactivity uptake areas (1) localized near sinusoids between hepatic parenchymal cords to one side of portal tributaries (2) (×100). c: The length of the "tracks" varies considerably, and it is difficult to see in the same image plane, hepatic architecture and tracks (×500). d: Nonspecific "grains" in hepatic lobule central region.

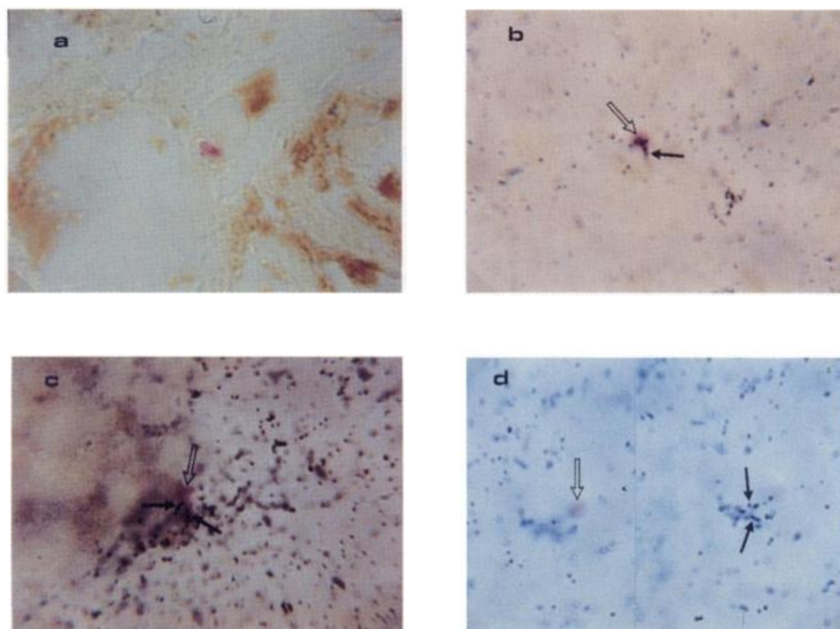

FIGURE 2
Kupffer cell phagocytosis of pink dye and ^{99m}Tc colloids. a: On non-autoradiographed slide, phagocytosis is located in Kupffer cells, after pink dye injection alone (×500). b and c: Pink dye particle (⇒ arrow) uptake associated with "tracks" (→ arrow), after simultaneous injection of pink dye and ^{99m}Tc colloids on MAR slides (×500). d: The same uptake area (dye (⇒ arrow) and ^{99m}Tc particles) on two different optical planes. Tracks (→ arrow) appeared gradually when modifying the focal plane of the microscope while pink spot disappeared.

In 1973, Chaudhuri and Coll (*15*) tried to compare the hepatic distribution of [^{99m}Tc]SC and [^{198}Au]colloid in mice by autoradiography. They observed major differences in the hepatic uptake of the two colloidal tracers. Colloidal gold showed pure intra Kupffer cell localization at 30 min following injection. On the other hand, colloidal ^{99m}Tc was evenly distributed within parenchymal and reticulo-endothelial cells. They suggested that the larger size of ^{99m}Tc-colloidal particles might prevent phagocytosis by Kupffer cells. However it is known that larger particles such as latex are easily phagocytosed (*5,14*).

We believe that the methodologic procedure can explain such differences. The emulsion (Kodak NTB), time of exposure, and developing procedure were identical for both radionuclides, thus neglecting the differences in energy characteristics and half-lives. However, the paraffin sections needed long preparation times (70 hr) which is a severely limiting factor when using ^{99m}Tc. Thus, the results obtained using the track method are in total discrepancy with the conclusions of Chaudhuri and Coll, but agree completely with the current knowledge of hepatic colloid phagocytosis by Kupffer cells. Indisputable evidence of such an uptake is given by (a) localization of tracks in the periphery of hepatic lobules in the area of hepatic sinusoids and (b) superimposition of dye colloidal drops and visible tracks.

However, one point deserves further discussion. The

correlation between ^{99m}Tc colloid and pink dye localization was not absolute, since some pink dye located in cells was unrelated to a track area. The average number of radioactivity areas seen on one hepatic lobule section was four, which is less than the expected number of Kupffer cells. The lack of visualization of radioactivity on all Kupffer cells may be due to the important variability in phagocytizing capacity between different Kupffer cells. It is probable that not all Kupffer cells participate in the uptake of the radioactive colloid.

CONCLUSION

"Track" micro autoradiography using a thick emulsion layer may be used on frozen tissue sections and provides an important tool in assessing the biodistribution of ^{99m} Tc-labeled radiopharmaceuticals.

Although highly predictable from physiological data, the hepatic uptake of ^{99m}Tc colloids by Kupffer cells has been questioned by other investigators. The current method provided direct evidence of Kupffer cell related uptake. The signal/noise ratio was high using the "track" method since tracks are clearly differentiated from background silver blackening. Therefore, semiquantitative measurements appear to be feasible. Intratissue modifications of the distribution versus time may be studied by this method, which may be relevant in the assessment of new ^{99m}Tc labeled drugs.

ACKNOWLEDGMENTS

The authors thank Drs. E. Rechenmann-Wittendorp and R. Rechenmann (University L. Pasteur, Strasbourg) for their help, comments, and suggestions.

REFERENCES

1. Wirquin E, Masse R, Meignan M, et al. Microautoradiography of gamma-emitters: interest of latensification progress. *Int J Appl Radiot Isot* 1984; 35:489–493.
2. Barbu M, Colas-Linhart N, Bok B. Technetium 99m autoradiography of labelled white cells. *Acta Haemat* 1984; 71:13–17.
3. Colas-Linhart N, Perianin A, Petiet A, et al. Sur une méthode permettant l'étude de la migration des leucocytes marqués par le technetium 99m. *CR Acad Sc Paris III* 1985; 10:413–416.
4. Wisse E, Knook DL. The investigation of sinusoidal cells: a new approach to the study of liver function. In: Popper H, Schaffner F, eds. *Progressive liver diseases*, Vol. 6. New York: 1979:153–167.
5. Buchanan JW, Wagner HN. Regional phagocytosis in man. In: *The reticuloendothelial system*, Vol 7B. New York: Plenum Publishing Corporation, 1985:247–270.
6. Rechenmann RV, Wittendorp E. Some basic and practical aspects on the development of nuclear emulsions. *J Microsc Biol Cell* 1976; 27:91–100.
7. Gavosto F, Rechenmann R. In vitro incorporation of glycine-1-^{14}C in reticulocytes. *Biochim Biophys Acta* 1954; 13:583–586.
8. Lysell L, Rohlin M. Initial Tc-99m diphosphonate uptake in mineralized and demineralized bone implants in rats. *Int J Oral Surg* 1985; 14:371–375.
9. Tomoike H, Nakamura M, Watanabe K, et al. Tc 99m PPi localization in acute experimental myocardial infarction: Application of macro and microautoradiography. *J Nucl Med* 1982; 23:84–85.
10. Tomoike H, Ogata I, Maruoka Y, et al. Differential registration of two types of radionuclides on macroautoradiograms for studying coronary circulation: concise communication. *J Nucl Med* 1983; 23:693–699.
11. Tilden RL, Jackson J, Williams J, et al. 99mTc polyphosphate: histological localization in human femurs in autoradiography. *J Nucl Med* 1973; 14:576–578.
12. Droz B. Artefacts in radioautography. *J Microsc Biol Cell* 1976; 27:133–134.
13. Lederer CM, Hollander JM, Perlman R. Table of isotopes. New York: John Wiley and Sons, 1978:423–426.
14. Afifi F, Sicot Ch, Benhamou JP, et al. Etudes sur la granulopexie. I- le phénomène de chasse. *Rev Franç Etudes Clin Biol* 1965; 10:79–83.
15. Chaudhuri TK, Evans TC, Chaudhuri TK. Autoradiographic studies of distribution in the liver of ^{198}Au and ^{99m}Tc-sulfur colloids. *Radiology* 1973; 109:633–637.